IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	VAN BRUGGEN ET AL.	Examiner:	HUNT, JENNIFER E.
Serial No.:	09/218,481	Group Art Unit:	1642
Filed:	DECEMBER 22, 1998	Docket No.:	11669.113US01
Title:	VASCULAR ENDOTHELIAL CELL GROWTH FACTOR ANTAGONISTS AND USES THEREOF		

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DECLARATION OF NICHOLAS VAN BRUGGEN UNDER 37 C.F.R. § 1.132

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Nicholas van Bruggen, Ph.D., of South San Francisco, CA, do declare and say as follows:

1. I am a research scientist employed by Genentech, Inc., and a co-inventor of the subject matter of U.S. Patent Application No. 09/218,481 filed December 22, 1998.

2. I understand that the Patent Examiner has rejected claims to the treatment of cerebral edema using an antagonist of VEGF as obvious in view of WO 94/10202, Ferrara et. al., published May 11, 1994 (Exhibit A); Criscuolo, 1994, *Yale J. Bio. Med.* 66:277-314 (Exhibit B); and Fischer et al., 1998 *Mol. Brain Res.* 60:98-99 (Exhibit C). I have read and understood these publications and the Office Action.

3. At the time of the invention, it was not publicly known or published that VEGF was a causative agent for cerebral edema or that an antagonist of VEGF could successfully treat cerebral edema *in vivo*. VEGF had been known for some time as a

permeablizing agent, but there were contradictory reports about the role of VEGF in edema. Various models provided correlative evidence of VEGF involvement in events surrounding edema. However, prior to the invention described in the present application, there was no direct evidence of a causative role for VEGF in cerebral edema, nor was there available a clinically effective treatment for cerebral edema using a VEGF antagonist. This is because, prior to the present invention, no suitable antagonist effective in a rodent model was available that could conclusively establish a direct causative role of VEGF in cerebral edema.

4. Contradictory evidence of the role of VEGF in cerebral edema existed in the literature at the time of the invention. In some correlative studies, the expression of VEGF mRNA in tumor cells may have been linked to pathologies other than edema formation, such as angiogenesis or mitogenesis. See, for example, Berkman et al., 1993, *J. Clin. Invest.*, 91:153-159 at page 157 (Exhibit D), where VEGF is identified as "VEGPF."

> [T]he correlation between VEGPF gene expression and tumor vascularity was stronger than was the correlation between VEGPF expression and cerebral edema. For example, capillary hemangioblastomas, which are one of the most vascular neoplasms in the CNS, exhibited the highest levels of VEGPF mRNA. However, these tumors are rarely associated with clinically significant cerebral edema. In contrast, metastatic tumors, which are not as vascular as capillary hemangioblastomas but are often associated with severe cerebral edema, had much lower levels of VEGPF mRNA compared to the hemangioblastomas. (Berkman et al. 1993 at page 157)

5. Hayashi et al. (1998, *J. Cereb. Blood Flow Metab.*, 18:887-895; Exhibit E) reported that VEGF itself, when applied topically to the surface of a reperfused rat brain after transient cerebral artery occlusion, reduced ischemic brain damage, infarct volume, and edema formation.

6. Studies, such as those correlating peritumoral edema formation with increased levels of VEGF mRNA expression in tumor cells while suggesting a role for VEGF in edema, presented no evidence of direct causation. More information was needed to conclude that VEGF itself induced cerebral edema. This position is adequately stated in the prior art. See, for example, Kalkanis et. al., 1996, *J. Neurosurg.* 85:1095-1101. (Exhibit F)

> Although these results suggest a possible role for VEGF in the widening of endothelial cell instricies to allow extravasation of plasma fluid into the adjacent peritumoral space, this study demonstrates only a strong correlation and not necessarily causation. (Kalkanis et al., 1996, at page 1099)

7. Although it was hypothesized that VEGF played a role in edema formation, this hypothesis could not be verified with certainty in the absence of an effective VEGF antagonist effective in a rodent model system. See, for example, Kalkanis, *supra* at page 1099.

> Although these results suggest a possible role for VEGF in the widening of endothelial cell instricies to allow extravasation of plasma fluid into the adjacent peritumoral space, this study demonstrates only a strong correlation and not necessarily causation. The ultimate proof for this hypothesis will require further studies involving VEGF blockade at one of several points along the cellular cascade and assessment of any subsequent resolution of peritumoral edema on MR imaging. (Kalkanis et al., 1996, at page 1099).

8. More recently, a study of peritumoral edema (PE) and angiogenesis of human glioblastoma tumors showed a clear correlation between tumor cell expression of VPF and tumor angiogenesis, but failed to show a significant correlation between the degree of peritumoral edema and the level of VPF expression. See Vaquero et al.,

2000, *J. Neuro-Oncology* 49:49-55 at page 49; Exhibit G). The authors of this study state (identifying VEGF as VPF) :

> Our present results suggest that in glioblastomas, tumor angiogenesis is clearly related to expression of VPF by tumor cells, but factors other than the intratumoral presence of VPF may contribute to the development of PE. (Vaquero et al., at page 49 in the summary).

9. Prior to the instant invention, I could not predict if VEGF was a causative agent of cerebral edema. See, for example, van Bruggen et al., 1999, *J. Clin. Invest.*, 104:1613-1620. (Exhibit H)

> To date, the role of VEGF in the pathophysiology of cerebral infarction remains unknown. Because the majority of research studies are performed on rodents, the lack of a suitable pharmacological antagonist effective in either rat or mouse prevents a clear understanding of the contribution of VEGF in the pathogenesis of stroke and related disorders. (van Bruggen et al., 1999, at page 1613)

10. At the time of the invention, I also could not predict that VEGF antagonists would be clinically effective for the treatment of cerebral edema. Because of the lack of a suitable pharmacological antagonist effective in animals models of the human disease, such as mouse or rat, it was unknown if the direct and specific elimination of VEGF by an antagonist would be sufficient to inhibit cerebral edema formation *in vivo*. See, for example, van Bruggen et al., *supra* at page 1613.

11. The anti-VEGF antibody A4.6.1 disclosed in WO 94/10202 specifically bound human VEGF. The antibody did not, however, neutralize the activity of rat VEGF. See, for example, Gossmann et al., 2002, *J. Magn. Reson. Imaging*, 15:233-240 at page 237 (Exhibit I). This aspect of A4.6.1 prevented its use in animal model studies in which endogenously expressed VEGF is important to the pathology.

12. The development of a VEGF antagonist effective in rat and mouse animal models allowed us to directly confirm a role for VEGF in cerebral edema formation. Ferrara et al. (1998, *Nat. Med.*, 4:336-340; Exhibit J) demonstrated truncated murine Flt-1 receptor fused to murine Fc-IgG as an effective inhibitor of rat and mouse VEGF *in vivo*. Using this fusion protein in the present invention to sequester VEGF *in vivo*, we demonstrated that VEGF is partly responsible for cortical edema formation in a rat model of ischemic injury with reperfusion. See van Bruggen et al. *supra* at page 1614.

13. The fusion protein allowed us to develop the first clinically effective treatment for cerebral edema utilizing a VEGF antagonist, as recited in the claims. See van Bruggen et al. *supra* at page 1614. Using the fusion protein to sequester VEGF *in vivo*, we demonstrated that antagonism of VEGF in a rat model of focal ischemic injury with reperfusion reduces cortical edema formation. See van Bruggen et al. *supra* at page 1614.

14. I understand the Patent Examiner asserts the corticosteroid dexamethasone is an antagonist of VEGF. At the time of the invention it was not publicly known or published that dexamethasone was an antagonist of VEGF. Corticosteriods were used for acute therapy of cerebral edema, although the mode of action was not certain. See van Bruggen et al. *supra* at page 1618.

15. Based on my understanding of the scientific literature, I do not consider dexamethasone to be an antagonist of VEGF. Antagonists of VEGF act by interfering with the binding of VEGF to a cellular receptor, by incapacitating or killing cells that have been activated by VEGF, or by interfering with vascular endothelial cell activation after VEGF binding to a cellular receptor. See Specification at page 7, lines 11-15. Scientific reports indicate coinjection of dexamethasone with VEGF or pretreatment with dexamethasone less than an hour prior to VEGF injection failed to alter the extent of vascular extravastion induced by VEGF alone in glioma and

endothelial cells. See Criscuolo *supra* at page 304. If dexamethasone were an antagonist of VEGF, one would have expected coinjection of dexamethasone with VEGF to decrease the extent of VEGF-induced vascular extravasation.

16. In addition, as discussed previously, contradictory reports exist in the literature about the role of VEGF in cerebral edema. In a later paper co-authored by Criscuolo, the relationship between VPF and peritumoral edema was studied in metastatic brain tumor tissue samples. See Strugar et al., 1994, *J. Neurosurg.*, 81:560; Exhibit K. VPF is essentially identical to VEGF. See Strugar et al., *supra* at page 564. The author reports that the study indicates that regardless of origin of the metastases, there is a high correlation between presence of VPF, vasogenic edema, and neovascularization. However, the authors go on to state that:

> However, although highly correlative, these observations do not prove that VPF is either the responsible agent for peritumoral brain edema and angiogenesis or that VPF represents a marker for metastatic potential. (Strugar et al., 1994, at page 565).

17. I understand the Patent Examiner asserts the barbiturates, methohexital and thiopental, are antagonists of VEGF. At the time of the invention it was not publicly known or published that methohexital and thiopental were antagonists of VEGF. As stated previously, antagonists of VEGF act by interfering with the binding of VEGF to a cellular receptor, by incapacitating or killing cells that have been activated by VEGF, or by interfering with vascular endothelial cell activation after VEGF binding to a cellular receptor. See Specification at page 7, lines 11-15.

18. As reported in the scientific literature, *in vitro* treatment of brain derived microvascular endothelial cells (BMEC) with VEGF and methohexital or thiopental did not reduce the permeability of the BMEC monolayer when compared to BMEC monolayers treated with VEGF only. See Fischer et al., *supra* at page 94, Figure 4. If methohexital or thiopental were antagonists of VEGF, one would have expected a

reduction in the VEGF-induced permeability of the BMEC monolayer. See Fischer et al., *supra* at page 94, Figure 4.

19. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and the like are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date: Dec 17 '02

Nicholas van Bruggen